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Ubiquitous parasites drive 33% increase in methane yield from livestock

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Abstract

Of anthropogenic methane emissions 40% can be attributed to agriculture, the majority of which are from enteric fermentation in livestock. With international commitments to tackle drivers of climate change, there is a need to lower global methane emissions from livestock production. Gastrointestinal helminths (parasitic worms) are globally ubiquitous and represent one of the most pervasive challenges to the health and productivity of grazing livestock. These parasites influence a number of factors affecting methane emissions including feed efficiency, nutrient use, and production traits. However, their effects on methane emissions are unknown. This is the first study that empirically demonstrates disease-driven increases in methane (CH₄) yield in livestock (grams of CH₄ per kg of dry matter intake). We do this by measuring methane emissions (in respiration chambers), dry matter intake (DMI), and production parameters for parasitised and parasite-free lambs. This study shows that parasite infections in lambs can lead to a 33% increase in methane yield (g CH₄/kg DMI). This knowledge will facilitate more accurate calculations of the true environmental costs of parasitism in livestock, and reveals the potential benefits of mitigating emission through controlling parasite burdens.

Key words: methane, greenhouse gas, climate change, parasites, disease; lambs

1. Introduction

Strategies for minimising global greenhouse gas (GHG) emissions from livestock systems are vital. Agriculture contributes an estimated 18% of GHG emissions (Steinfeld et al., 2006), with approximately half of these emissions coming from meat and dairy (Garnett, 2009). Emissions are a particular concern in small ruminant (sheep and goat) milk and meat production as 56% of the global domestic ruminant population are small ruminants (Marino et al., 2016), and enteric fermentation is responsible for the majority of emissions in these systems (Gerber et al., 2013). Minimising GHG emissions from livestock systems will become increasingly important as demand for livestock products grows; by 2050 the global sheep population is expected to increase by 60%, from 1.7 billion in 2000 to about 2.7 billion by 2050 (Foresight, 2011). With little chance of decreasing emissions through an overall reduction in the numbers of farmed ruminants, other ways to mitigate ruminant methane emissions are required (Herrero et al., 2016; Dangal et al., 2017).

The primary factors affecting ruminant enteric methane emissions are thought to be feed intake levels, feed composition, and the microflora of the rumen (Lascano & Cárdenas, 2010). Consequently, mitigation options currently fall into three broad categories: 1) animal breeding for improved efficiency; 2) feed supplements and feed management; and 3) rumen control and modifiers (Marino et al., 2016). However, a number of these strategies have high costs and/or inconsistent effects (Marino et al., 2016) and reliable, affordable technologies for reducing methane emissions in grazing livestock in a way that improves overall farm productivity and efficiency remain elusive.

Gastrointestinal helminths are globally ubiquitous, offering the most pervasive challenge to all grazing livestock worldwide and compromising animal health, welfare and production efficiency. They have a substantial impact on the majority of the factors affecting methane production, including feed intake levels, nutrient use, and rumen retention time (Houdijk et al., 2016). Controlling gastrointestinal parasites could potentially reduce GHG emissions in grazing livestock. However, their effects on methane emissions are currently unknown.

In addition to revealing the mitigation potential of reducing parasitism, understanding the impact of parasitism on methane production is also vital in calculating the true extent of GHG emissions from livestock. Many studies have attempted to calculate the GHG emissions from livestock systems, often with the aim of quantifying how changes in efficiency will impact on methane production (Kipling et al., 2016; Özkan et al., 2016). Emissions estimates are generally calculated based on livestock numbers, time in the production system, and basic national multipliers. However, such calculations ignore the potential impacts of common infections. Efforts have been made to explore the impacts of parasitism on production efficiency and time on pasture, and the consequent implications for emissions (Kenyon et al., 2013). However this approach assumes that methane yield remains constant regardless of infection status, and that parasitism has no additional effect beyond the higher overall feed intake due to decreased production efficiency and increased time to slaughter.

By quantifying how parasitism affects methane emissions per unit of feed intake, we can obtain a more complete understanding of the environmental costs of parasitism and the potential benefits of mitigating emission through controlling parasite burden. Here, we address this aim by

evaluating methane emissions per unit of feed intake in parasitised and non-parasitised finishing lambs, using respiration chambers.

2. Materials and methods

The protocol was conducted under Home Office licence (PPL 60/4489) and was approved by SRUC's Animal Experiment Committee (AE ED 24-2015).

2.1 Animals and experimental design

A total of 72 parasite naïve lambs (Suffolk x Mule), 12-15 weeks old were selected from a commercial sheep flock. All animals were expected to be parasite naïve at the start of the trial, as they were reared indoors and only fed commercial pelleted feed. Their parasite free status was confirmed using faecal egg counts. The animals were divided into three treatment groups, balanced for live weight (mean body weight at day 0 = 36.62kg \pm 0.35 S.E.) and sex (mixed pens). These treatments were: *Ad lib* fed control ; restricted-fed control ; and parasitised .

There were a total of eight replicates for each treatment, with each replicate comprised of one pen of three lambs. There were three lambs in each pen to ensure adequate eructation for methane detection. The lambs were housed in indoor pens in these groups of three for the duration of the trial. The trial lasted for 39 days and animals were returned to stock at the end of the trial.

2.2 Parasite challenge

The animals in the parasitised treatment were trickle challenged with 7,000 infective *Teladorsagia circumcincta* larvae suspended in 10ml of water, three times a week from days 0 to 35 (five weeks). *T. circumcincta* is an abomasal nematode which represents a substantial parasitic challenge to sheep, and is often linked with parasitic gastroenteritis in lambs (Coop et al., 1982). The trickle infection was used to represent the challenge encountered by grazing lambs, and was expected to result in subclinical infection consistent with rates of natural infection on moderately parasitised pasture (Coop et al., 1982). The *ad lib* control, and restricted-fed control treatments were sham infected with 10ml of water, following the same protocol as the parasitised treatment. Parasite levels were monitored through weekly faecal sampling for faecal egg counts (FEC), using the modified flotation method with a sensitivity of 1 egg per gram of faeces (epg)(Christie & Jackson, 1982). To give an indication of gut damage by *T.circumcincta*, pepsinogen levels were measured from blood samples taken at three points in the trial. Blood samples were collected from all animals at day 0 (pre-challenge), day 36 (peak challenge, prior to being placed in the respiration chambers), and day 39 (after removal from the respiration chambers).

2.3 Feeding

The *ad-lib* control and parasitised treatments were fed *ad-lib* access to pelleted grass. The restricted fed treatments were fed 80% of the intake of their *ad lib* fed counterparts, relative to body weight. Parasite induced anorexia was expected in the parasitised treatment, hence the restricted-fed control group enabled the assessment of the impact of parasitism *per se* versus that of anorexia associated with parasitism. All lambs were fed their rations once a day.

2.4 Measurements

2.4.1 Methane

From days 43 to 46 lambs were housed in indirect open-circuit respiration chambers (No Pollution Industrial Systems Ltd., UK). The trial was conducted over four rounds, using six respiration chambers, with treatments balanced across each round so that each treatment was tested in two respiration chambers per round. The area of each chamber is 25.4m² with penning for three lambs. Temperature and humidity were set at 15 ± 1°C and 60 ± 5% respectively, and air was removed from the chambers by exhaust fans set at 50litres/s. Methane concentration was recorded for the air in each chamber once every six minutes, using infrared absorption spectroscopy. Animals remained in the chambers for three full days (days 43 to 46), the first 24 hours were the adaptation period, and measurements taken during the final 48 hours (days 44-46) were used to quantify methane production. Total feed intake in the chambers was measured daily, and methane yield (g CH₄/kg DMI) was calculated by dividing daily methane production by the daily DMI.

2.4.2 Digestibility

The collection of faeces directly from the rectum of all lambs was carried out over three consecutive days (days 30 to 32), pooled per lamb, and stored at -20°C prior to digestibility analysis. Acid insoluble ash (AIA) was used as an internal, indigestible marker to assess the apparent total tract digestive matter (DM). Faecal and feed AIA were analysed using the 2N HCl

procedure (Van Keulen & Young, 1977). During feeding, feed samples were collected daily and pooled for analysis.

2.4.3 Feed intake and weight gain

Pelleted feed intake was measured three times a week, to calculate the restricted-feeding allowances. Feed intake was also measured daily in each respiration chamber for calculation of methane yield (gCH₄/kg DMI). All lambs were weighed weekly.

2.5 Statistical analysis

Data were analysed using ANOVA, with round as the block term. For statistics pertaining to body weight, day 0 body weight was included as a covariate. Daily intake values are presented per animal, by dividing the total pen intake by three. All statistical analyses were performed in GENSTAT 16

3. Results

3.1 Development of the parasite challenge

Lamb FEC increased over time for the parasitised treatment, indicating that parasite infections were achieved in the groups dosed with *T. circumcincta*, whilst the control and restricted-fed control treatments remained parasite free for the duration of the trial (Fig. 1a). Pepsinogen levels were significantly higher in the parasitised group at the two sample points in the final week of the trial ($P<0.001$) (Fig. 1b). These increased levels of blood pepsinogen confirmed abomasal damage in the parasitised animals compared to the controls. The FEC and pepsinogen results indicate that the parasitised treatment group did harbour helminth infection when in the respiration chambers, whilst the *ad lib* and restricted fed control groups did not. No clinical signs of parasitism were observed in any groups throughout the experiment.

3.2 Lamb performance

Table 1 shows the variation in performance, feed intake, and digestibility, across the three treatment groups. The DMI of the parasitised group was significantly lower than the *ad lib* group ($P<0.001$), indicative of parasite induced anorexia with their feed intake being an average of approximately 80% of the *ad lib* control group over the study period. Maximum anorexia was found in the final week of the study, where average daily DMI per animal in the parasitised group was approximately 70% of the *ad lib* control group. The highest level of inappetance coincided with the time of highest FEC. Average body weight gain was 174 g/day in control *ad lib* fed individuals, whilst the average body weight gain for *ad lib* fed parasitised individuals was 7 g/day.

3.3 Methane output and yield

Methane output was significantly higher in the *ad lib* fed control group ($P < 0.001$), than in the other two treatments (Fig. 2). Whilst methane emissions remained relatively steady over time in the two *ad lib* fed treatments (*ad lib* control and parasitised), in the restricted fed group the emissions rose steadily shortly after feeding time, before reaching a peak and declining again.

Although total methane emissions were highest in the *ad lib* fed control group, Fig. 3 reveals that methane yield ($\text{g CH}_4/\text{kg DMI}$) was significantly higher in the parasitised group. Methane yield was 33% higher in the parasitised lambs compared to the *ad lib* control group. Whilst there was a significant difference in methane yield between the parasitised treatment group and both control treatment groups ($P < 0.001$), there was no significant difference in methane yield between the *ad lib* fed control group and the restricted-fed control group despite a significant difference in feed intake (Fig. 3).

4. Discussion

This study aimed to quantify the impact of parasitism on methane emissions in lambs. Our results show that methane yield was 33% higher in the parasitised lambs relative to the *ad lib* control group. This is the first study to demonstrate that infectious disease can increase methane yield (g CH₄/kg DMI).

The total quantity of methane produced per day was highest in the *ad lib* control group (Fig. 2). This is because the primary driver of methane production (g CH₄/day) is dry-matter intake (DMI), with a strong positive correlation between methane emissions and DMI (Buddle et al., 2011). The *ad lib* control group had a significantly higher level of DMI ($P < 0.001$), providing a higher total supply of substrate for methane production in the rumen. Whilst in the respiration chambers the parasitised lambs consumed 70% of the feed quantity consumed by the *ad lib* control group. This reduced intake was associated with 20% less methane production in the parasitised animals. Snap shot measurements of methane output would therefore show parasitism being associated with a positive environmental impact. However, the methane yield (g CH₄/kg DMI) was 33% higher in the parasitised animals compared to the *ad-lib* control group. The parasitised lambs also had significantly lower weight gain compared to the controls, and would require a higher overall feed intake over their lifetime to reach target weight. Whilst worldwide there is a mixture of sheep management practices i.e. intensively and extensively reared lambs and a variety of different nutritional environments, parasite induced anorexia is a phenomena which occurs over all systems where livestock are at risk from gastrointestinal parasites (Kyriazakis *et al.* 1998; Sutherland & Scott, 2010). Thus the combination of increased methane

yield, and higher feed intake per kg product demonstrated in this study has substantial implications for the impacts of parasitism on emissions from meat production.

Low feed intake can be associated with increased methane yield, however, the methane yield from the parasitised animals was higher than would be expected based solely on their lower DMI (Hammond et al., 2013). Additionally, despite a significant difference in DMI between the *ad lib* and restricted fed control groups, there was no significant difference in methane yield between these groups (Table 1 and Fig. 3). These findings suggest that parasitism has an impact on methane yield beyond that expected from changes in DMI alone. The extent of bacterial fermentation is influenced by myriad elements of gastrointestinal physiology and digesta kinetics (Moraes et al., 2014; Stergiadis et al., 2016). Gastrointestinal nematode infection in small ruminants can lead to substantial changes in the digestive tract including increased cell turnover, changes in permeability, changes in pH, altered secretory activities (e.g. mucous production), and inhibited gastric acid production (Li et al., 2016; Louie et al., 2007). Some of these parasite induced changes in the gastrointestinal tract will disrupt the intricate interactions between hosts and their gut microbiome, as the large array of products secreted by gastrointestinal nematodes impact on growth and metabolism of resident microbial communities (Zaiss & Harris, 2016). However, we are only now beginning to understand the complexity of microbiota, and the effects of parasitism on interactions between hosts and their gastrointestinal bacteria remain largely unexplored (Buddle et al., 2011; Zaiss & Harris, 2016). Thus the effects of parasitism on microbial survival, proliferation, spatial organisation, and ultimately rate of methanogenesis, are yet to be understood. Whilst our results identify a novel phenomenon, they do not reveal the mechanism.

In this study, weight gain was significantly lower in the parasitised group compared to that in other groups. This highlights the substantial impact of parasitism on productivity, with parasitised hosts needing to stay in the system much longer to reach slaughter weight. Attempts have previously been made to quantify the impacts of parasitism on emissions through exploring the increased time on pasture, and increased DMI required to reach slaughter weight. Without accounting for the effects of parasitism on emissions per kg DMI such studies will likely underestimate the full influence of parasitism on methane production. The parasite driven increase in methane yield demonstrated in this study, combined with the knowledge that parasitism decreases production efficiency and increases time to achieve production targets (Houdijk et al., 2016; Kenyon et al., 2013), demonstrates that parasitism has the potential to have substantial impacts on livestock methane emissions. In addition to emissions increasing with parasitism is the concern that parasite intensity is projected to increase under climate change (Fox et al., 2011, 2012, 2015).

The potential impact that parasitism has on livestock emissions makes it an attractive target for mitigation. Parasite control practices (i.e. rearing indoors, clean grazing and *refugia*-based control strategies), which break the parasite lifecycle, provide an opportunity to sustainably reduce GHG emissions as it is cost effective, practical, and improves overall production efficiency. As the increase in ovine meat production is expected to be highest in developing countries (O'Mara, 2011), with restricted access to improved feeds, feed supplements and efficiency gains through genetic selection, parasite control offers a viable and accessible way of reducing emissions.

273

274 This study shows that parasite infections in lambs can lead to a 33% increase in methane yield.
275 Combined with impacts of parasitism on production efficiency, and the subsequent increased
276 time on pasture, there is potential for parasitism to have an extensive impact on GHG emissions.
277 There are international commitments to reduce GHG emissions, and an informed understanding
278 of how production-limiting diseases affect GHG production is vital in developing public policies
279 and combating climate change. As we improve our understanding of how parasitism affects
280 livestock methane emissions we begin to elucidate the true environmental costs of parasitism,
281 and reveal the potential benefits of mitigating emission through controlling infectious disease.

282

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289

290 **Declarations of interest**

291 None

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Figure legends

Figure 1. Indirect measures of parasitism across all treatment groups.

A) Mean faecal egg counts (FEC) (eggs/g faeces) by trial week (\pm SE), and B) mean pepsinogen levels (\pm SE) at three time points, for all three treatment groups of lambs - Ad lib control (*ad lib* fed), restricted fed control (fed 80% of feed intake of *ad lib* control, to account for parasite induced anorexia), and parasitised lambs (also *ad lib* fed) .

Figure 2. Daily mean methane output

Mean methane output in A) grams per hour (\pm SE), and B) grams per day (\pm SE), for Ad lib control (*ad lib* fed), restricted fed control (fed 80% of feed intake of *ad lib* control, to account for parasite induced anorexia), and parasitised lambs (also *ad lib* fed), averaged across individuals.

Figure 3. Mean Methane yield

Mean methane yield (grams of methane per kg of dry matter intake) (\pm SE) for Ad lib control (*ad lib* fed), restricted fed control (fed 80% of feed intake of *ad lib* control, to account for parasite induced anorexia), and parasitised lambs (also *ad lib* fed).

Table 1. Performance, feed intake and digestibility

The mean body weight parameters, levels of feed intake, and digestibility values for *ad lib* control lambs, restricted fed control lambs, and parasitised lambs, averaged across individuals.

Values in rows with different letter superscripts differed significantly ($P < 0.05$).

	Treatments				P-value
	<i>Ad lib</i> control	Restricted fed control	Parasitised	Standard error	
Final BW (kg)	42.6 ^a	37.1 ^b	38.2 ^b	0.8	<0.001
BW gain (g/day) per animal	174 ^a	71 ^b	7 ^c	12.8	<0.001
Daily DMI over trial, per animal (g/day)	1783 ^a	1302 ^b	1396 ^c	28.3	<0.001
Daily DMI per kg BW over trial (g/kg BW/day)	44.6 ^a	34.4 ^b	37.0 ^c	0.98	<0.001
Digestibility Dry Matter (DM, %)	55.4	58.2	58.4	0.01	0.09

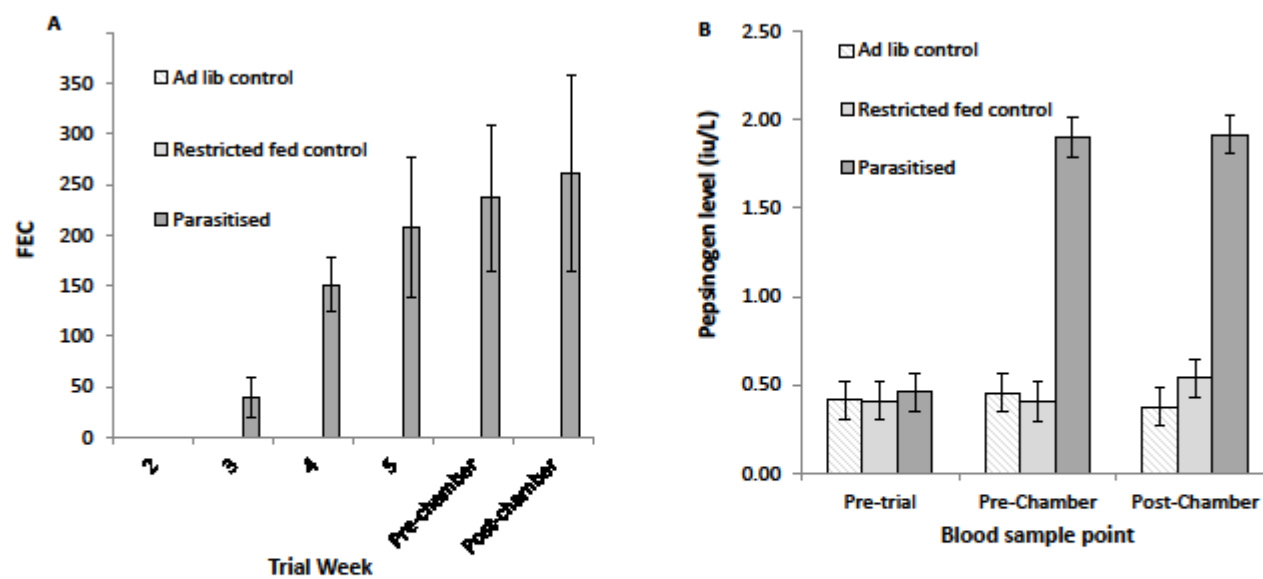


Figure 1.

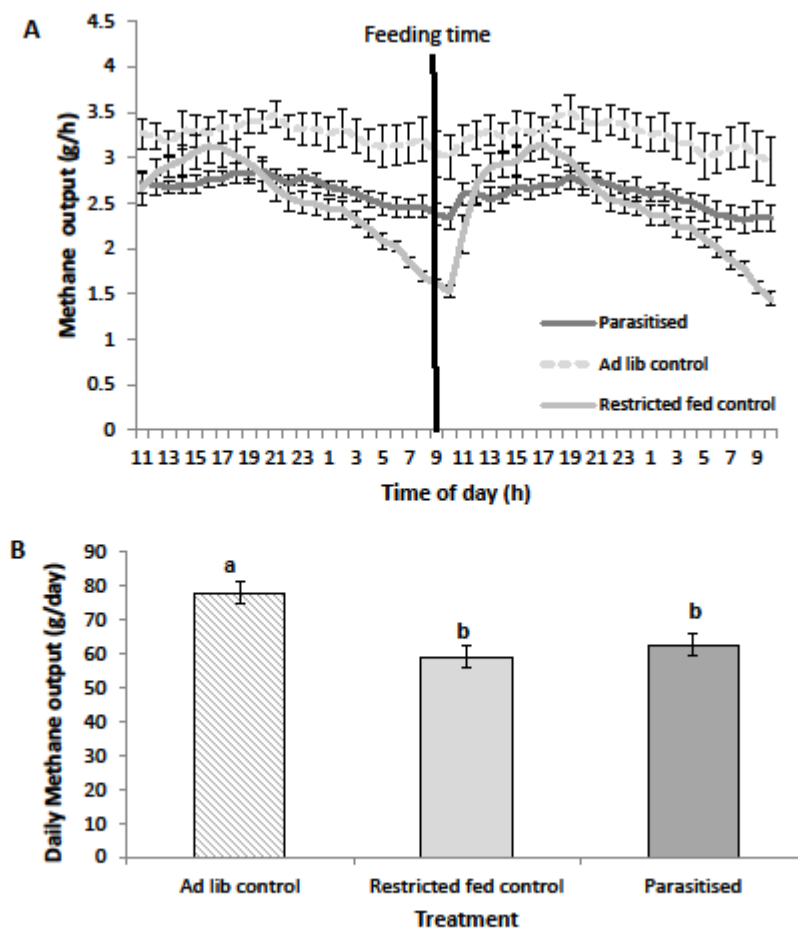


Figure 2.

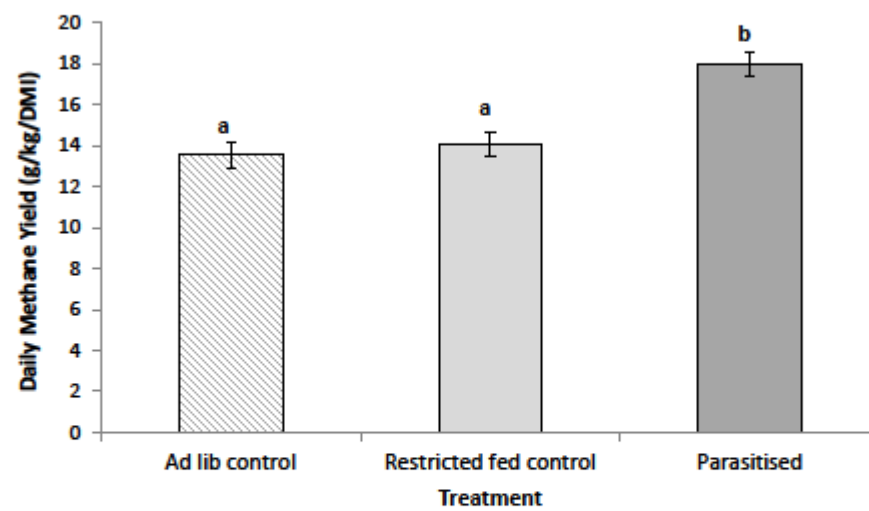


Figure 3.